EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	71	sarcosine adj oxidase and proline	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:28
L2	48	sarcosine adj oxidase and proline and mutant	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:28
L3	108	v94	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:29
L4	0	l2 and l3	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:29
L5	9	val94	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:30
L6	0	val94 and l1	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:30
L7	42	l1 and valine and glycine	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:31
L8	36	l1 and valine and glycine and mutant	US-PGPUB; USPAT; DERWENT	OR	ON	2006/05/17 10:31

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NEWS	7	FEB 27	New STN AnaVist pricing effective March 1, 2006
NEWS	8	MAR 03	Updates in PATDPA; addition of IPC 8 data without attributes
NEWS	9	MAR 22	EMBASE is now updated on a daily basis
NEWS	10	APR 03	New IPC 8 fields and IPC thesaurus added to PATDPAFULL
NEWS	11	APR 03	Bibliographic data updates resume; new IPC 8 fields and IPC thesaurus added in PCTFULL
NEWS	12	APR 04	STN AnaVist $500 visualization usage credit offered
NEWS	13	APR 12	LINSPEC, learning database for INSPEC, reloaded and enhanced
NEWS	14	APR 12	Improved structure highlighting in FQHIT and QHIT display in MARPAT
NEWS	15	APR 12	Derwent World Patents Index to be reloaded and enhanced during second quarter; strategies may be affected
NEWS	16	MAY 10	CA/CAplus enhanced with 1900-1906 U.S. patent records
NEWS	17	MAY 11	KOREAPAT updates resume

NEWS EXPRESS FEBRUARY 15 CURRENT VERSION FOR WINDOWS IS V8.01a,
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=> s sarcosine (w) oxidase and mutant and proline
L1 10 SARCOSINE (W) OXIDASE AND MUTANT AND PROLINE

=> d ibib abs l1 1-10

L1 ANSWER 1 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2005676631 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16363800
TITLE: Ionization of zwitterionic amine substrates bound to monomeric **sarcosine oxidase**.
AUTHOR: Zhao Gouhua; Jorns Marilyn Schuman
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102, USA.
CONTRACT NUMBER: GM 31704 (NIGMS)
SOURCE: Biochemistry, (2005 Dec 27) Vol. 44, No. 51, pp. 16866-74. Journal code: 0370623. ISSN: 0006-2960.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200604
ENTRY DATE: Entered STN: 22 Dec 2005
Last Updated on STN: 22 Apr 2006
Entered Medline: 21 Apr 2006

AB Monomeric **sarcosine oxidase** (MSOX) binds the L-**proline** zwitterion (pKa = 10.6). The reactive substrate anion is generated by ionization of the ES complex (pKa = 8.0). Tyr317 was mutated to Phe to determine whether this step might involve proton transfer to an active site base. The mutation does not eliminate the ionizable group in the ES complex (pKa = 8.9) but does cause a 20-fold decrease in the maximum rate of the reductive half-reaction. Kinetically determined Kd values for the ES complex formed with L-**proline** agree with results obtained in spectral titrations with the wild-type or **mutant** enzyme. Unlike the wild-type enzyme, Kd values with the **mutant** enzyme are pH-dependent, suggesting that the mutation has perturbed the pKa of a group that affects the Kd. As compared with the wild-type enzyme, an increase in charge transfer band energy is observed for **mutant** enzyme complexes with substrate analogues while a 10-fold decrease in the charge transfer band extinction coefficient is found for the complex with the L-**proline** anion. The results eliminate Tyr317 as a possible acceptor of the proton released upon substrate ionization. Since previous studies rule out the only other nearby base, we conclude that L-**proline** is the ionizable group in the ES complex and that amino acids are activated for oxidation upon binding to MSOX by stabilization of the reactive substrate anion. Tyr317 may play a role in substrate activation and optimizing binding, as judged by the effects of its mutation on the observed pKa, reaction rates, and charge transfer bands.

L1 ANSWER 2 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2002398214 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12146941
TITLE: Monomeric **sarcosine oxidase**: role of histidine 269 in catalysis.
AUTHOR: Zhao Gouhua; Song Hui; Chen Zhi-Wei; Mathews F Scott; Jorns Marilyn Schuman
CORPORATE SOURCE: Department of Biochemistry, MCP Hahnemann School of Medicine, Philadelphia, PA 19129, USA.
CONTRACT NUMBER: GM 31611 (NIGMS)
GM 31704 (NIGMS)
SOURCE: Biochemistry, (2002 Aug 6) Vol. 41, No. 31, pp. 9751-64.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1L9C; PDB-1L9D; PDB-1L9E
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 31 Jul 2002
Last Updated on STN: 6 Sep 2002
Entered Medline: 5 Sep 2002

AB Conservative mutation of His269 (to Asn, Ala, or Gln) does not significantly affect the expression of monomeric **sarcosine oxidase** (MSOX), covalent flavinylation, the physicochemical properties of bound FAD, or the overall protein structure. Turnover with sarcosine and the limiting rate of the reductive half-reaction with L-**proline** at pH 8.0 are, however, nearly 2 orders of magnitude slower than that with with wild-type MSOX. The crystal structure of the His269Asn complex with pyrrole-2-carboxylate shows that the pyrrole ring of the inhibitor is displaced as compared with wild-type MSOX. The His269

mutants all form charge-transfer complexes with pyrrole-2-carboxylate or methylthioacetate, but the charge-transfer bands are shifted to shorter wavelengths (higher energy) as compared with wild-type MSOX. Both wild-type MSOX and the His269Asn **mutant** bind the zwitterionic form of L-**proline**. The E(ox).L-**proline** complex formed with the His269Asn **mutant** or wild-type MSOX contains an ionizable group (pK(a) = 8.0) that is required for conversion of the zwitterionic L-**proline** to the reactive anionic form, indicating that His269 is not the active-site base. We propose that the change in ligand orientation observed upon mutation of His269 results in a less than optimal overlap of the highest occupied orbital of the ligand with the lowest unoccupied orbital of the flavin. The postulated effect on orbital overlap may account for the increased energy of charge-transfer bands and the slower rates of electron transfer observed for **mutant** enzyme complexes with charge-transfer ligands and substrates, respectively.

L1 ANSWER 3 OF 10 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:188047 BIOSIS
DOCUMENT NUMBER: PREV200600182129
TITLE: Ionization of zwitterionic amine substrates bound to monomeric **sarcosine oxidase**.
AUTHOR(S): Zhao, Gouhua; Jorns, Marilyn Schuman [Reprint Author]
CORPORATE SOURCE: Drexel Univ, Coll Med, Dept Biochem and Mol Biol, Philadelphia, PA 19102 USA
marilyn.jorns@drexelmed.edu
SOURCE: Biochemistry, (DEC 27 2005) Vol. 44, No. 51, pp. 16866-16874.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Mar 2006
Last Updated on STN: 15 Mar 2006

AB Monomeric **sarcosine oxidase** (MSOX) binds the L-**proline** zwitterion (pK(a) = 10.6). The reactive substrate anion is generated by ionization of the ES complex (pK(a) = 8.0). Tyr317 was mutated to Phe to determine whether this step might involve proton transfer to an active site base. The mutation does not eliminate the ionizable group in the ES complex (pK(a) = 8.9) but does cause a 20-fold decrease in the maximum rate of the reductive half-reaction. Kinetically determined K-d values for the ES complex formed with L-**proline** agree with results obtained in spectral titrations with the wild-type or **mutant** enzyme. Unlike the wild-type enzyme, K-d values with the **mutant** enzyme are pH-dependent, suggesting that the mutation has perturbed the pK(a) of a group that affects the K-d. As compared with the wild-type enzyme, an increase in charge transfer band energy is observed for **mutant** enzyme complexes with substrate analogues while a 10-fold decrease in the charge transfer band extinction coefficient is found for the complex with the L-**proline** anion. The results eliminate Tyr317 as a possible acceptor of the proton released upon substrate ionization. Since previous studies rule out the only other nearby base, we conclude that L-**proline** is the ionizable group in the ES complex and that amino acids are activated for oxidation upon binding to MSOX by stabilization of the reactive substrate anion. Tyr317 may play a role in substrate activation and optimizing binding, as judged by the effects of its mutation on the observed pK(a), reaction rates, and charge transfer bands.

L1 ANSWER 4 OF 10 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:489027 BIOSIS
DOCUMENT NUMBER: PREV200200489027
TITLE: Monomeric **sarcosine oxidase**: Role of histidine 269 in catalysis.
AUTHOR(S): Zhao, Gouhua; Song, Hui; Chen, Zhi-wei; Mathews, F. Scott;

Jorns, Marilyn Schuman [Reprint author]
CORPORATE SOURCE: Department of Biochemistry, MCP Hahnemann School of Medicine, Philadelphia, PA, 19129, USA
marilynjorns@drexel.edu
SOURCE: Biochemistry, (August, 2002) Vol. 41, No. 31, pp. 9751-9764. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 18 Sep 2002
Last Updated on STN: 18 Sep 2002

AB Conservative mutation of His269 (to Asn, Ala, or Gln) does not-significantly affect the expression of monomeric **sarcosine oxidase** (MSOX), covalent flavinylation, the physicochemical properties of bound FAD, or the overall protein structure. Turnover with sarcosine and the limiting rate of the reductive half-reaction with L-**proline** at pH 8.0 are, however, nearly 2 orders of magnitude slower than that with with wild-type MSOX. The crystal structure of the His269Asn complex with pyrrole-2-carboxylate shows that the pyrrole ring of the inhibitor is displaced as compared with wild-type MSOX. The His269 **mutants** all form charge-transfer complexes with pyrrole-2-carboxylate or methylthioacetate, but the charge-transfer bands are shifted to shorter wavelengths (higher energy) as compared with wild-type MSOX. Both wild-type MSOX and the His269Asn **mutant** bind the zwitterionic form of L-**proline**. The EoxcntdotL-**proline** complex formed with the His269Asn **mutant** or wild-type MSOX contains an ionizable group (pKa = 8.0) that is required for conversion of the zwitterionic L-**proline** to the reactive anionic form, indicating that His269 is not the active-site base. We propose that the change in ligand orientation observed upon mutation of His269 results in a less than optimal overlap of the highest occupied orbital of the ligand with the lowest unoccupied orbital of the flavin. The postulated effect on orbital overlap may account for the increased energy of charge-transfer bands and the slower rates of electron transfer observed for **mutant** enzyme complexes with charge-transfer ligands and substrates, respectively.

L1 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:1257631 CAPLUS
DOCUMENT NUMBER: 144:65851
TITLE: Ionization of Zwitterionic Amine Substrates Bound to Monomeric **Sarcosine Oxidase**
AUTHOR(S): Zhao, Gouhua; Schuman Jorns, Marilyn
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, 19102, USA
SOURCE: Biochemistry (2005), 44(51), 16866-16874
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Monomeric **sarcosine oxidase** (MSOX) binds the L-**proline** zwitterion (pKa = 10.6). The reactive substrate anion is generated by ionization of the ES complex (pKa = 8.0). Tyr317 was mutated to Phe to determine whether this step might involve proton transfer to an active site base. The mutation does not eliminate the ionizable group in the ES complex (pKa = 8.9) but does cause a 20-fold decrease in the maximum rate of the reductive half-reaction. Kinetically determined Kd values for the ES complex formed with L-**proline** agree with results obtained in spectral titrns. with the wild-type or **mutant** enzyme. Unlike the wild-type enzyme, Kd values with the **mutant** enzyme are pH-dependent, suggesting that the mutation has perturbed the pKa of a group that affects the Kd. As compared with the wild-type enzyme, an increase in charge transfer band energy is observed for **mutant**

enzyme complexes with substrate analogs while a 10-fold decrease in the charge transfer band extinction coefficient is found for the complex with the L-**proline** anion. The results eliminate Tyr317 as a possible acceptor of the proton released upon substrate ionization. Since previous studies rule out the only other nearby base, we conclude that L-**proline** is the ionizable group in the ES complex and that amino acids are activated for oxidation upon binding to MSOX by stabilization of the reactive substrate anion. Tyr317 may play a role in substrate activation and optimizing binding, as judged by the effects of its mutation on the observed pKa, reaction rates, and charge transfer bands.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:430956 CAPLUS
DOCUMENT NUMBER: 141:3265
TITLE: Engineered **sarcosine oxidase** **mutant** with improved stability and less reactive to **proline**, as creatine assay reagent
INVENTOR(S): Kishimoto, Takahide; Sogabe, Atsushi; Oka, Masanori
PATENT ASSIGNEE(S): Toyo Boseki Kabushiki Kaisha, Japan
SOURCE: PCT Int. Appl., 54 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004044193	A1	20040527	WO 2003-JP14423	20031113
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
JP 2004159565	A2	20040610	JP 2002-329427	20021113
JP 2004159566	A2	20040610	JP 2002-329428	20021113
JP 2004242526	A2	20040902	JP 2003-33641	20030212
AU 2003284548	A1	20040603	AU 2003-284548	20031113
EP 1561812	A1	20050810	EP 2003-774008	20031113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
US 2006051832	A1	20060309	US 2005-534583	20050511

PRIORITY APPLN. INFO.:

JP 2002-329427	A	20021113
JP 2002-329428	A	20021113
JP 2003-33641	A	20030212
WO 2003-JP14423	W	20031113

AB Modified **sarcosine oxidase** having improved stability in the liquid state compared with the unmodified one and/or having a lowered activity on L-**proline** compared with the unmodified one and having an excellent substrate specificity without losing the **sarcosine oxidase** activity, and use as creatine assay reagent, are disclosed. A process for producing **sarcosine oxidase** which comprises culturing a microorganism capable of producing **sarcosine oxidase** and collecting the **sarcosine oxidase** from the culture medium; is also claimed. Such modified **sarcosine oxidase** have at

least one of the following characteristics, i.e., an activity on L-**proline** being 0.7% or less based on sarcosine and a Km value to L-**proline** being 150 mM or more, when measured at 37° and pH 8.0;. A **mutant** of Arthrobacter strain TE1826 **sarcosine oxidase** was prepared by substituting various residues to reduce its reactivity to Pro, which causes errors during the determination of creatine or creatine in body fluid. The Pro reactivity of the **mutants** was reduced by >70%.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:523961 CAPLUS
DOCUMENT NUMBER: 137:212826
TITLE: Monomeric **Sarcosine Oxidase**: Role of Histidine 269 in Catalysis
AUTHOR(S): Zhao, Gouhua; Song, Hui; Chen, Zhi-wei; Mathews, F. Scott; Schuman Jorns, Marilyn
CORPORATE SOURCE: Department of Biochemistry, MCP Hahnemann School of Medicine, Philadelphia, PA, 19129, USA
SOURCE: Biochemistry (2002), 41(31), 9751-9764
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Conservative mutation of His269 (to Asn, Ala, or Gln) does not-significantly affect the expression of monomeric **sarcosine oxidase** (MSOX), covalent flavinylation, the physicochem. properties of bound FAD, or the overall protein structure. Turnover with sarcosine and the limiting rate of the reductive half-reaction with L-**proline** at pH 8.0 are, however, nearly 2 orders of magnitude slower than that with wild-type MSOX. The crystal structure of the His269Asn complex with pyrrole-2-carboxylate shows that the pyrrole ring of the inhibitor is displaced as compared with wild-type MSOX. The His269 **mutants** all form charge-transfer complexes with pyrrole-2-carboxylate or methylthioacetate, but the charge-transfer bands are shifted to shorter wavelengths (higher energy) as compared with wild-type MSOX. Both wild-type MSOX and the His269Asn **mutant** bind the zwitterionic form of L-**proline**. The Eox·L-**proline** complex formed with the His269Asn **mutant** or wild-type MSOX contains an ionizable group (pKa = 8.0) that is required for conversion of the zwitterionic L-**proline** to the reactive anionic form, indicating that His269 is not the active-site base. We propose that the change in ligand orientation observed upon mutation of His269 results in a less than optimal overlap of the highest occupied orbital of the ligand with the lowest unoccupied orbital of the flavin. The postulated effect on orbital overlap may account for the increased energy of charge-transfer bands and the slower rates of electron transfer observed for **mutant** enzyme complexes with charge-transfer ligands and substrates, resp.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:613512 CAPLUS
DOCUMENT NUMBER: 129:241780
TITLE: Preparation of **sarcosine oxidase mutant** less reactive to **proline**
INVENTOR(S): Nishiya, Yoshiaki; Kawamura, Yoshiharu
PATENT ASSIGNEE(S): Toyobo Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 10248572	A2	19980922	JP 1997-55203	19970310
PRIORITY APPLN. INFO.:			JP 1997-55203	19970310

AB A **sarcosine oxidase mutant** is prepared from the **sarcosine oxidase** of Arthrobacter strain TE1826 by substituting 345-Phe with Ala, Gly, Val, or Ile to reduce its reactivity to Pro, which causes errors during the determination of creatine or creatinine in body fluid. The **mutant** enzyme exhibits a pH optimum 7.5-8.5, temperature optimum 40-50°, and mol. weight 43 kDa by SDS-PAGE. The Pro reactivity of the **mutants** is reduced by >70%. Claimed is a creatine/creatinine assay reagent composition comprised of the **sarcosine oxidase mutant** and other reagents.

L1 ANSWER 9 OF 10 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2006001834 EMBASE
TITLE: Ionization of zwitterionic amine substrates bound to monomeric **sarcosine oxidase**.
AUTHOR: Zhao G.; Jorns M.S.
CORPORATE SOURCE: M.S. Jorns, Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102, United States. marilyn.jorns@drexelmed.edu
SOURCE: Biochemistry, (27 Dec 2005) Vol. 44, No. 51, pp. 16866-16874. .
Refs: 20
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 26 Jan 2006
Last Updated on STN: 26 Jan 2006

AB Monomeric **sarcosine oxidase** (MSOX) binds the L-**proline** zwitterion (pK (a) = 10.6). The reactive substrate anion is generated by ionization of the ES complex (pK(a) = 8.0). Tyr317 was mutated to Phe to determine whether this step might involve proton transfer to an active site base. The mutation does not eliminate the ionizable group in the ES complex (pK (a) = 8.9) but does cause a 20-fold decrease in the maximum rate of the reductive half-reaction. Kinetically determined K(d) values for the ES complex formed with L-**proline** agree with results obtained in spectral titrations with the wild-type or **mutant** enzyme. Unlike the wild-type enzyme, K(d) values with the **mutant** enzyme are pH-dependent, suggesting that the mutation has perturbed the pK(a) of a group that affects the K (d). As compared with the wild-type enzyme, an increase in charge transfer band energy is observed for **mutant** enzyme complexes with substrate analogues while a 10-fold decrease in the charge transfer band extinction coefficient is found for the complex with the L-**proline** anion. The results eliminate Tyr317 as a possible acceptor of the proton released upon substrate ionization. Since previous studies rule out the only other nearby base, we conclude that L-**proline** is the ionizable group in the ES complex and that amino acids are activated for oxidation upon binding to MSOX by stabilization of the reactive substrate anion. Tyr317 may play a role in substrate activation and optimizing binding, as judged by the effects of its mutation on the observed pK(a), reaction rates, and charge transfer bands. .COPYRGT. 2005 American Chemical Society.

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ACCESSION NUMBER: 2002278510 EMBASE
TITLE: Monomeric **sarcosine oxidase**: Role of histidine 269 in catalysis.
AUTHOR: Zhao G.; Song H.; Chen Z.-W.; Mathews F.S.; Jorns M.S.
CORPORATE SOURCE: M.S. Jorns, Department of Biochemistry, MCP Hahnemann School of Medicine, Philadelphia, PA 19129, United States. marilynjorns@drexel.edu
SOURCE: Biochemistry, (6 Aug 2002) Vol. 41, No. 31, pp. 9751-9764.
.
Refs: 29
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
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AB Conservative mutation of His269 (to Asn, Ala, or Gln) does not-significantly affect the expression of monomeric **sarcosine oxidase** (MSOX), covalent flavinylation, the physicochemical properties of bound FAD, or the overall protein structure. Turnover with sarcosine and the limiting rate of the reductive half-reaction with L-**proline** at pH 8.0 are, however, nearly 2 orders of magnitude slower than that with with wild-type MSOX. The crystal structure of the His269Asn complex with pyrrole-2-carboxylate shows that the pyrrole ring of the inhibitor is displaced as compared with wild-type MSOX. The His269 **mutants** all form charge-transfer complexes with pyrrole-2-carboxylate or methylthioacetate, but the charge-transfer bands are shifted to shorter wavelengths (higher energy) as compared with wild-type MSOX. Both wild-type MSOX and the His269Asn **mutant** bind the zwitterionic form of L-**proline**. The E(ox).ovrhdot.L-**proline** complex formed with the His269Asn **mutant** or wild-type MSOX contains an ionizable group (pK(a) = 8.0) that is required for conversion of the zwitterionic L-**proline** to the reactive anionic form, indicating that His269 is not the active-site base. We propose that the change in ligand orientation observed upon mutation of His269 results in a less than optimal overlap of the highest occupied orbital of the ligand with the lowest unoccupied orbital of the flavin. The postulated effect on orbital overlap may account for the increased energy of charge-transfer bands and the slower rates of electron transfer observed for **mutant** enzyme complexes with charge-transfer ligands and substrates, respectively.